

# Association between coefficients of variation of the R-R intervals on electrocardiograms and post-challenge hyperglycemia in patients with newly diagnosed type 2 diabetes

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## ABSTRACT

The aim of the present study was to examine whether there is a relationship between autonomic function and post-challenge hyperglycemia in patients with type 2 diabetes. Subjects included 122 Japanese patients newly diagnosed with type 2 diabetes. Autonomic nerve function was assessed using coefficients of variation of the R-R intervals on electrocardiograms (CVRR). Unlike anthropometry, insulin secretion and insulin resistance, age ( $r = -0.209$ ,  $P < 0.021$ ) and post-challenge plasma glucose at 120 min (PG120;  $r = -0.219$ ,  $P < 0.015$ ) were the only variables significantly correlated with CVRR. Age was not significantly correlated with PG120. In multiple regression analyses, CVRR Z-score, but not age, was significantly correlated with PG120. The present results suggest that autonomic function affects post-challenge blood glucose levels independently of age. (*J Diabetes Invest*, doi: 10.1111/j.2040-1124.2010.00098.x, 2011)

**KEY WORDS:** Type 2 diabetes mellitus, Hyperglycemia, Autonomic function

## INTRODUCTION

Diabetic autonomic neuropathy is a major complication in patients with diabetes; it decreases quality of life and increases mortality. Long-term hyperglycemia is a primary cause of diabetic neuropathy<sup>1</sup>. Conversely, autonomic function might affect glycemic control through gastrointestinal peristalsis. In addition, results from animal experiments have suggested that the autonomic nervous system contributes to glucose homeostasis by mediating interorgan networks<sup>2-4</sup>. Matsuhisa *et al.* have reported that the vagus nerve, which in part controls the liver, plays an important role in regulating postprandial glucose levels<sup>5,6</sup>. In addition, the intestinal-brain-liver neuronal axis has been reported to be involved in liver gluconeogenesis<sup>4</sup>. However, evidence showing that these pathways and autonomic function play a role in glycemic control is lacking in humans. The aim of the present study was to examine whether there is a relationship between autonomic function, evaluated by coefficient of variation of the R-R interval on electrocardiograms (ECG; CVRR), and post-challenge hyperglycemia in patients with newly diagnosed type 2 diabetes.

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## MATERIAL AND METHODS

### Subjects

A total of 122 Japanese patients newly diagnosed with type 2 diabetes mellitus between January 2008 and December 2009, in the Division of Endocrinology and Metabolism at Kanazawa University Hospital, were recruited for the present study. None of the patients were treated with an oral hypoglycemic agent or insulin. Table 1 shows the demographic, clinical and physical characteristics of the subjects.

Written informed consent was obtained from all patients before initiation of the study. The study was approved by the ethics committee established at the Kanazawa University Hospital (Approval No. 729) and was carried out in accordance with the Declaration of Helsinki.

### Laboratory Data

HbA<sub>1c</sub> was described in the Japan Diabetes Society (JDS) value. Oral glucose tolerance tests using 75 g of glucose (75-g OGTT) were carried out for all patients. The plasma glucose concentration was measured using an automated glucose analyzer (model GA08; ATWiLL M.I., Kanazawa, Japan). The plasma insulin concentration was measured by immunoassay (AIA-1800ST; Tosoh, Tokyo, Japan).

Insulin resistance in the liver was evaluated using the liver insulin resistance index (L-IR) as reported by Muhammad *et al.*<sup>7</sup>. Insulin resistance in muscle was evaluated using the muscle

**Table 1** | Clinical characteristics of the 74 newly diagnosed type 2 diabetic patients

Clinical parameters	Value	
Age (years)	60.1 ± 11.1	
Sex (male/female)	82/40	
Body mass index (kg/m <sup>2</sup> )	25.1 ± 4.7	
Fasting plasma glucose (mg/dL)	139.9 ± 31.8	
HbA <sub>1c</sub> (%) (JDS)	7.9 ± 2.0	
Diabetic complications	<i>n</i>	Prevalence (%)
Retinopathy (Fukuda†)		
0	88/112	79
A1	7/112	6
A2	6/112	5
A3	4/112	3
B1	2/112	2
B4	5/112	4
Nephropathy		
Stage 1	90/120	75
Stage 2	19/120	16
Stage 3a	7/120	6
Stage 3b	3/120	3
Stage 4	1/120	1
Somatic polyneuropathy‡	17/95	18

Data are means ± SD. JDS, Japan Diabetes Society. †Retinopathy stages were diagnosed according to Fukuda's classification (Fukuda M. Classification and treatment of diabetic retinopathy. *Diabetes Research and Clinical Practice*. 1994; 24 Suppl: s171–s176) as follows: Stage 0, patients without retinopathy; Stage A1, mild to moderate simple retinopathy; Stage A2, severe simple retinopathy; Stage A3, mild to moderate interrupted proliferative retinopathy; Stage A4, 5, severe interrupted proliferative retinopathy; Stage B1, pre-proliferative diabetic retinopathy; Stage B2, early proliferative diabetic retinopathy; Stage B3, advanced proliferative diabetic retinopathy; Stage B4, end-stage proliferative diabetic retinopathy. ‡Somatic polyneuropathy was defined as below mean-2SD in nerve conduction velocity in two or more of six sites in the healthy subject.

insulin resistance index (M-IR) as reported by Matsuda *et al.*<sup>8</sup>. To evaluate initial insulin secretion, the insulinogenic index was calculated by dividing the change in immunoreactive insulin (IRI) over 30 min by the change in plasma glucose (PG) over 30 min:  $\Delta\text{IRI} (30' - 0') \mu\text{U/mL} / \Delta\text{PG} (30' - 0') \text{mg/dL}$ .

### Evaluation of Autonomic Nerve Function

The CVRR was used to evaluate diabetic autonomic neuropathy<sup>9</sup>. After the patient had rested in the supine position for at least 10 min, a standard 12-lead ECG was recorded (Cardio Star FCP-7541; Nihon Kohden, Tokyo, Japan). The R-R intervals were measured for 3 min, and the CVRR was obtained by dividing the standard deviations (SD) by the means (M):  $\text{CV} (\%) = (\text{SD}/\text{M}) \times 100$ . Because CVRR is influenced by age, we also used CVRR Z-score calculated by  $(\text{CVRR} - \text{mean CVRR}) / \text{SD-CVRR}$  in each age category to the age-specific normal value of CVRR (Table S2).

### Statistical Analysis

For statistical analyses, SPSS II for Windows (SPSS, Chicago, IL, USA) was used. Single regression analysis and multiple regression analysis were used to examine associations between CVRR and clinical parameters. All data are presented as means ± SD.

### RESULTS

Clinical characteristics of the 122 newly diagnosed type 2 diabetic patients are shown in Table 1. The prevalence of retinopathy and nephropathy was 21% and 25% of the subjects, respectively. When somatic polyneuropathy is defined as below mean-2SD in nerve conduction velocity in more than two of six sites in the healthy subjects, its prevalence was 18%.

The results for single linear regression analyses between CVRR and each clinical parameter are shown in Table 2. CVRR was significantly correlated with age ( $r = -0.209$ ,  $P < 0.021$ ) and PG120 ( $r = -0.219$ ,  $P < 0.015$ ; Table 2), but was not correlated with body mass index, HbA<sub>1c</sub>, insulinogenic index, PG0-60 and IRI0-120, L-IR, and M-IR ( $P > 0.05$ ; Table 2). Although CVRR diminishes with age, CVRR, but not age, significantly correlated to PG120 (Table S1). In a multiple regression analysis for PG 120 as a dependent variable, CVRR was still significantly correlated with PG120 ( $P = 0.004$ ), even after adjustment for age (Table 3, upper panel). When we used CVRR Z-scores in the analysis, CVRR Z score, but not age, was significantly correlated with PG120 in a multiple regression model (Table 3, lower panel).

**Table 2** | Single linear regression analyses between coefficients of variation of the R-R intervals on electrocardiograms and clinical parameters

Variable	<i>r</i> -value	<i>P</i> -value
Age	-0.209	0.021*
BMI	0.188	0.075
HbA <sub>1c</sub> (JDS)	0.057	0.536
Insulinogenic index	0.014	0.902
PG0	-0.054	0.554
PG30	0.041	0.650
PG60	-0.108	0.236
PG120	-0.219	0.015*
IRI0	-0.114	0.211
IRI30	-0.021	0.817
IRI60	0.002	0.978
IRI120	0.024	0.794
L-IR	0.042	0.718
M-IR	-0.182	0.121

\* $P < 0.05$ . PG0, plasma glucose concentration before load; PG30, plasma glucose concentrations at 30 min after load; PG60, plasma glucose concentrations at 60 min after load; PG120, plasma glucose concentrations at 120 min after load; IRI0, immunoreactive insulin before load; IRI30, immunoreactive insulin at 30 min after load; IRI60, immunoreactive insulin at 60 min after load; IRI120, immunoreactive insulin at 120 min after load; L-IR, liver insulin resistance index; M-IR, muscle insulin resistance index.

**Table 3** | Multiple regression analysis for plasma glucose concentrations at 120 min after load as a dependent variable and clinical parameters as independent variables

	Partial regression coefficient $\beta$	t-value	P-value
Sex	-12.061	-1.135	0.259
Age	-1.109	-1.994	0.048*
CVRR	-16.879	-2.901	0.004*
Sex	-12.091	-1.143	0.255
Age	-0.534	-0.924	0.357
CVRR Z-score	-18.123	-2.935	0.004*

CVRR, coefficients of variation of the R-R intervals on electrocardiograms.

\* $P < 0.05$ ;  $n = 121$ .

## DISCUSSION

The preset results suggest that autonomic nerve dysfunction, specifically parasympathetic nerve dysfunction, increases post-challenge glucose levels without affecting insulin secretion or insulin sensitivity. One possible mechanism underlying autonomic dysfunction-associated post-challenge hyperglycemia is gastrointestinal peristalsis that affects absorption of nutrients<sup>10</sup>.

The failure of liver gluconeogenesis, regulated by the vagus nerve, has been shown to contribute to diabetic autonomic neuropathy-related hyperglycemia. Wang *et al.*<sup>4</sup> reported that lipids in the upper intestine activate the intestine-brain-liver neural axis to inhibit glucose production. This mechanism, which inhibits liver gluconeogenesis after food consumption, is mediated through the vagus nerve, which innervates the small intestine from the cerebrum. We suspect that dysfunction of this mechanism caused by diabetic autonomic neuropathy might contribute to hyperglycemia.

The neuronal pathway might mediate the action of incretin hormones secreted by the gut, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent gastric inhibitory peptide (GIP). Incretin hormones enhance glucose-mediated insulin secretion and suppress exaggerated glucagon secretion<sup>11-14</sup>, thereby regulating postprandial plasma glucose levels.

Diabetic autonomic neuropathy might also cause the dysfunction of cardiovascular, gastrointestinal, genitourinary, sudomotor or ocular organs<sup>1</sup>. Postprandial and post-challenge hyperglycemia is an independent risk factor for macrovascular disease<sup>15-17</sup> as well as many other complications, such as diabetic retinopathy<sup>18</sup>, increased carotid intima-media thickness<sup>19</sup>, increased oxidative stress<sup>20</sup>, decreased myocardial blood volume and myocardial blood flow<sup>21</sup>, increased risk for cancer<sup>22</sup>, and impaired cognitive function<sup>23</sup>. Further studies are needed to clarify whether diabetic autonomic neuropathy increases the risks for these complications.

In conclusion, the present results suggest that autonomic function affects post-challenge blood glucose levels independently of age in patients with type 2 diabetes.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1** | Single linear regression analyses between PG120 and clinical parameters

**Table S2** | Age-specific normal values of the coefficients of variation of the R-R interval on electrocardiograms (CVRR)

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